10/036444

CJE

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on April 18, 2006.

Frank C Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. INN-103TD1
Patent No. 6,979,546

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Alessandro Moretta, Cristina Bottino, Roberto Biassoni
Issued	:	December 27, 2005
Patent No.	:	6,979,546
For	:	Triggering Receptor Involved in Natural Cytotoxicity Mediated by Human Natural Killer Cells and Antibodies That Identify the Same

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 9, line 63:	Page 16, lines 14-15:
"anti-p44 antibodies"	--anti-NKp44 antibodies--

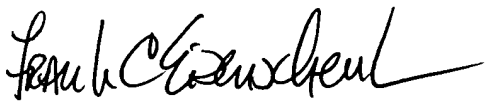
Column 13, line 25:	Page 22, line 21:
"Fcy"	--Fcγ--
Column 18, line 60:	Page 32, line 17:
"cuvefte"	--cuvette--
Column 22, line 35:	Page 38, lines 29-30:
"AZ**20** cell"	--AZ20$^-$ cell--
Column 25, line 7:	Page 43, line 13:
"EMBUGenBank"	--EMBL/GenBank--
Column 25, line 66:	Page 44, line 28:
"NKp46 associated CD**34**ζ chain"	--NKp46-associated CD3ζ chain--
Column 26, line 43:	Page 46, line 3:
"(CD**3**ζFcεRIγ and"	--(CD3ζ/FcεRIγ and--
Column 26, line 61:	Page 46, line 16:
"NKp46/NKp44 independent"	-- NKp46/NKp44-independent--
Column 27, line 19:	Page 47, line 4:
"CD**34** ζ"	-- CD3ζ--
Column 27, line 21:	Page 47, line 5:
"CD**34**ζ"	-- CD3ζ--
Column 37, line 22:	Amendment dated August 10, 2004 (original claim 74, renumbered as claim 1):
"NK cells wvith"	--NK cells with--

Column 37, line 23:	Amendment dated August 10, 2004 (original claim 74, renumbered as claim 1):
"cffmdve"	--effective--
Column 37, line 25:	Amendment dated August 10, 2004 (original claim 74, renumbered as claim 1):
"specificely"	--specifically--
Column 38, line 27:	Amendment dated February 28, 2005 (original claim 85, renumbered as claim 7):
"Registrati"	--Registration--
Column 38, line 36:	Amendment dated August 10, 2004 (original claim 93, renumbered as claim 10):
"fragnent"	--fragment--

A true and correct copy of pages 16, 22, 32, 38, 43, 44, 46, and 47 of the specification as filed and Applicants' Amendments dated August 10, 2004 and February 28, 2005 which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/lkw/gyl
Attachments: Copy of pages 16, 22, 32, 38, 43, 44, 46, and 47 of the specification
Amendments dated August 10, 2004 and February 28, 2005

(*e.g.* saturating concentration) that said regulation more strongly balances in favor of NK cell cytotoxicity stimulation.

The stimulating method of the invention does advantageous not require the conventional steps of NK cell incubation in interleukines such as IL-2, IL-12, IL-15. These steps are however of course not precluded: the skilled person nevertheless can as desired choose to add these conventional steps to the method of the invention. The present invention also relates to any kit for stimulating NK cell cytotoxicity, comprising at least one of said products enclosed in a container.

For detection, quantification, removal, positive purification, and/or NK cell cytotoxicity stimulation, said methods of the invention can further comprise contacting said biological sample, or said NK cells with an anti-NKp46 or anti-NKp44 antibodies; and said kits can further comprise anti-NKp46 or anti-NKp44 antibodies.

Advantageously, the purifying method of the invention can simultaneously perform NK cell activation, and *vice versa*. This simultaneous NK cell positive purification and NK cell cytotoxicity stimulation embodiment of the invention is of particular interest when applied to biological samples, and particularly to samples deriving from human person(s) and meant to be re-administered to a human patient after treatment.

Alternatively, the present invention also provides with a method for inhibiting NK cell cytotoxicity, comprising contacting said NK cells under physiological conditions with at least one compound:

(a) capable of inhibiting the binding of NKp30 natural ligands to NKp30 receptors expressed on said NK cells, *e.g.* by masking NKp30 binding sites, and/or capable of inhibiting the cross-linking of the NKp30 receptors expressed by said NK cells, and/or

(b) capable of inhibiting the interactions between the NKp30 molecules expressed by said NK cells, and their transduction elements, notably CD3ζ.

NKp30 ligand expressed by a NK-susceptible target cell, and the comparison this measured level to the standard physiological one. This assessment is of special interest for the diagnostic of tumor cells and/or microorganism-infected cells, and prescription of appropriate prevention, palliation, therapy tools.

These and other features and advantages of the invention will be further apparent from the following examples. These examples are given for illustrative purposes only, and are in no way intended to restrict the scope of the present invention. Alternative embodiments, intended by any skilled person, are encompassed by the present invention.

Description of the drawings

In these examples, reference is made to figures 1A to 9B (19 drawing sheets):

- Figures 1A, 1B, 1C illustrate the triggering of NK-mediated cytolytic activity induced by three mAbs according to the invention (anti-NKp30 mAbs).

On figure 1A, a representative polyclonal NK cell population was analyzed (% specific ^{51}Cr release) for cytolytic activity in a redirected killing assay against the Fcγ R-positive P815 target cell in the absence or in the presence of c127 (anti-CD16), BAB281 (anti-NKp46), Z231 (anti-NKp44), AZ20, A76, Z25 (anti-NKp30 mAbs) and c218 (anti-CD56) mAbs. The E/T (effector:target) ratio used was 1:1. The AZ20 hybridoma (CNCM Registration Number I-2576) has been accepted for deposit at the Collection Nationale De Cultures De Micro-organismes (CNCM) Institute Pasteur, 28, rue du Dr. Roux, 757 Paris Cèdex 15, France on November 8, 2000 under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms.

APR 27 2006

On figure 1B, the representative NK clone 3M16 was analyzed (% specific ^{51}Cr release) in a redirected killing assay against P815 target cells

micrograms/ml for the redirected killing experiments. The E/T ratios are indicated in the text. Appropriate mAbs include those which significantly increase the cytolytic activity observed in their absence. Examples of such an appropriate significant increase comprise an increase of at least about 5 times of the cytolytic activity observed with an effector: target ratio of 1:1 in the presence of said mAbs when compared to the cytolytic activity observed in the absence of these mAbs.

Determination of intracellular free calcium [Ca++]i increase

Determination of [Ca++]i was performed as previously described (Poggi A., R. Pardi, N. Pella, L. Morelli, S. Sivori, M. Vitale, V. Revello, A. Moretta, and L. Moretta. 1993. CD45-mediated regulation of LFA1 function in human natural killer cells. Anti-CD45 monclonal antibodies inhibit the calcium mobilization induced via LFA1 molecules. *Eur. J. Immunol.* 23:2445-2463). Fura-2-labeled NK cells were incubated for 30' at 4°C with saturating amounts of anti-NKp30 mAb (AZ20) or medium alone. Cross-linking of this receptor was obtained by adding into the cuvette 20 μg/ml of affinity purified Goat Anti-Mouse antiserum (GAM) (ICN Biomedicals, Aurora, Ohio).

Biochemical characterization of the NKp30 molecules

Integral NK cell membrane proteins (Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* 256:1604-1606) were prepared as follows: $25x10^6$ cells were lysed in 100 μl TX buffer (20 mM Sodium phosphate buffer, 1% Triton X-114, 10 mM EDTA, pH 8) 30' at 4°C, centrifuged (5', 10.000 RPM). The supernatant was left 10' at 37°C, centrifuged and lower phase was resuspended 1:2 in TX buffer and left 10' at 4°C in order to clarify the lysates. The suspension was then left 10' at 37°C, centrifuged and the lower phase resuspended 1:3 in EB (0.0625 M Tris pH6.8, 10% Glycerol, 2.3% SDS). Samples were analyzed in discontinuous SDS-PAGE, transferred to Immobilon P (Millipore Corp,

was also detected with EBV-induced B cell lines, monocytic and DC lines, and different hemopoietic and non-hemopoietic tumor cell lines including HL60, U937, Eo/A3, THP-1, Daudi, Jurkat, IGROV and all the various tumor cell lines that were used as target cells.

We recently showed that polyclonal NK cell populations from some donors were characterized by a bimodal distribution of fluorescence intensity of NKp46 molecules ($NKp46^{bright}$ and $NKp46^{dull}$) and that NK clones derived from these individuals expressed a stable $NKp46^{bright}$ or $NKp46^{dull}$ phenotype. Importantly, the cytolytic activity of NK cell clones against NK susceptible target cells strictly correlated with their NKp46 phenotype. We then analyzed the reactivity of the new mAbs on polyclonal NK cell populations and NK cell clones derived from individuals displaying different patterns of NKp46 expression. As shown in fig.2A, the polyclonal NK cell population derived from the representative donor AM displayed a homogeneously bright phenotype when stained by either AZ20 or anti-NKp46 mAbs. On the contrary, in the polyclonal NK cells derived from donor CB, staining with the same mAbs resulted in a bimodal distribution of fluorescence. Notably, in donor CB the same pattern of fluorescence intensity was also detectable in fresh purified NK cells (fig.2A). Moreover, the analysis of several clones derived from donor CB, revealed that $NKp46^{bright}$ clones were consistently $AZ20^{bright}$, whereas $NKp46^{dull}$ clones always displayed an $AZ20^{dull}$ phenotype (fig.2B).

In order to further define the pattern of reactivity of the new mAbs in freshly isolated lymphocytes, PBL derived from different individuals were assessed by double fluorescence analysis using informative mAbs. A representative donor is shown in fig.3A: the surface molecule recognized by AZ20 mAb was selectively expressed on $CD56^+$ cells. Moreover most $AZ20^+$ cells co-expressed CD16 molecules. On the other hand, AZ20 mAb did not stain $CD3^+$ T lymphocytes or $HLA\text{-}DR^+$ B lymphocytes. It is of note that the $CD56^+$ $AZ20^-$ cell population detected in this donor also expressed surface CD3 molecules. Therefore, also in freshly derived lymphocytes, the reactivity of AZ20 mAb

signal peptide of 18 amino acid (SEQ ID N°3) and by an extracellular region of 120 amino acids (SEQ ID N°4) forming an Ig-like domain of the V-type. The extracellular portion contains two potential N-linked glycosylation sites and no consensus sequences for O-linked glycosylation. A region rich in hydrophobic amino acids, potentially involved in protein-protein interactions, is connecting the Ig V-like domain with the transmembrane region. The 19 amino acid transmembrane region (SEQ ID NO: 5) contains the positively charged amino acid, Arg and the 33 amino acid cytoplasmic portion (SEQ ID NO: 6) lacks typical ITAM consensus sequences. The presence of a charged amino acid in the transmembrane domain is a feature common to other triggering receptors expressed on NK cells. These charged residues are usually thought to be involved in the association with ITAM containing signaling polypeptides.

EMBL/GenBank databases searching revealed that the clone 5C cDNA (SEQ ID NO: 1) was 76.8% identical to a previously identified alternatively spliced form of the 1C7 gene (Acc. NO: AF031138). This gene has been mapped on human chromosome 6, in the TNF cluster of MHC gene complex (Nalabolu, S.R., H. Shukla, G. Nallur, S. Parimoo and S.M. Weissman. 1996. Genes in a 220-kb region spanning the TNF cluster in human MHC. *Genomics* 31:215-22). So far however, neither the function nor the surface distribution of the putative product of 1C7 gene could be identified; and no mAb specific to 1C7 was available. Moreover, the 1C7 transcript could not be revealed by Northern blot on different tissues and cell lines. On the other hand, by RT-PCR the 1C7 transcript could be amplified by RNA isolated from spleen (but not from other tissues) or certain lymphoid and myeloid cell lines. These data suggested that 1C7 transcripts could be poorly represented or could be expressed at substantial levels only in a narrow range of cell types. Our present analysis of NKp30 expression by Northern blotting revealed a mRNA of approximately 1kb in polyclonal NK cell populations and NK cell lines including NKL and NK3.3. On the contrary, consistent with the lack of reactivity with anti-NKp30 mAbs, no NKp30 mRNA could be detected in human monocytes or cell lines of different histotype including U937, Jurkat,

HL60 and LCL 721.221 cells (fig.8A). In some of these cell lines which were negative for mRNA expression by Northern blot (and for anti-NKp30 mAb surface staining) it has been possible to detect transcripts when analyzed by RT-PCR technique. This finding is likely to reflect a low level of NKp30 transcription resulting in lack of NKp30 surface expression. Moreover, Northern blot analysis of multiple human tissues showed selective expression of NKp30 transcript only in spleen. Altogether these data are consistent with the fact that NKp30 expression is largely NK-specific.

Finally, the human NKp30 cDNA probe hybridized with genomic DNA from monkey, rat, mouse, dog, cow and rabbit. These data support the fact that the NKp30 encoding gene is highly conserved in different species (fig.8B).

Biochemical characterization of the NKp30 complex

A NKp30-specific antiserum was generated by immunizing rabbits with an N-terminal NKp30 peptide. As shown in fig.9A, the antiserum recognized in Western blot a molecule identical to that previously detected by AZ20 mAb. Unlike the AZ20 mAb, the antiserum immunoprecipitates NKp30 molecules from polyclonal NK cell populations labeled with biotin. Thus, a polyclonal NK cell population, treated or not with sodium pervanadate, was immunoprecipitated with the NKp30-specific antiserum and probed with anti-phosphotyrosine mAb. In order to avoid non specific binding of rabbit Immunoglobulin to CD16 molecules, cell lysates were extensively precleared with anti-CD16 mAb. Moreover, in all experiments pre-immune rabbit serum was used as negative control. In these experiments no tyrosine phosphorylation of NKp30 receptor could be detected. On the other hand, NKp30 receptor associated with a molecule that became tyrosine phosphorylated upon sodium pervanadate treatment (fig.9B) and co-migrated with the NKp46-associated CD3ζ chain. The identity between the NKp30-associated molecule and CD3ζ polypeptides was directly demonstrated by its reactivity with anti-CD3ζ mAb (fig.9B).

of tumor targets. Both belong to the Ig superfamily but do not display significant identity. They associate to different signal transducing polypeptides (CD3ζ/FcεRIγ and KARAP/DAP12, respectively) that become tyrosine phosphorylated upon NK cell activation. NKp46 and NKp44 were shown to co-operate in the process of tumor cell lysis by human NK cells.

However, lysis of certain target cells was only marginally NKp46- and/or NKp44-dependent since mAb-mediated masking of these molecules did not significantly interfere with cytotoxicity. Moreover although clearly NKp46- and/or NKp44-dependent, the cytolytic activity against other tumor cell lines could not be abrogated by mAb-mediated masking of both molecules suggesting again the existence of additional receptor(s) co-operating with NKp46 and NKp44. Indeed, we show here that NKp30 represents a receptor that may co-operate with NKp46 and NKp44 in the induction of cytotoxicity against a variety of target cells. Perhaps, more importantly, NKp30 represents the major receptor in inducing NK-mediated killing of certain tumor target cells the lysis of which is largely NKp46/NKp44-independent (*e.g.* melanoma of the MEL 15 type). Remarkably, NKp30, similar to NKp46, is also involved in NK cell activation and target cell killing by fresh NK cells.

As discussed above, the surface expression of NKp30 parallels that of NKp46. Indeed, NK cells displaying a $NKp46^{dull}$ or a $NKp46^{bright}$ phenotype, were also characterized by $NKp30^{dull}$ or $NKp30^{bright}$ fluorescence. We previously showed that NK cell clones characterized by a $NKp46^{dull}$ phenotype consistently express low amounts of NKp44. The finding that NK cells express parallel densities of different triggering receptors may explain the existence of NK cell subsets displaying different "natural" cytolytic activity. For example, it was difficult to understand why the cytolytic activity against some target cells (such as MEL15) although largely NKp46-independent, was essentially confined to NK clones expressing the $NKp46^{bright}$ phenotype. These results can now be explained by the finding that only $NKp46^{bright}$ cells express high density of NKp30 receptor. Thus, the previous demonstration of major differences in cytolytic activity of $NKp46^{dull}$ and $NKp46^{bright}$ cells can now

be applied also to NK cells displaying different NKp30 phenotypes. Along this line, the cytolytic activity of NKp30^{dull} NK cell clones was markedly reduced as compared to that of NKp30^{bright} clones.

NKp30, similar to NKp46, associates with CD3ζ that is most likely involved in signaling via the receptor complex. However, CD3ζ does not appear to be necessary for the surface expression of both receptors at least in COS-7 cells. Molecular cloning revealed that NKp30 is the product of 1C7, a gene previously mapped on human chromosome 6 in the HLA class III region (Nalabolu, S.R., H. Shukla, G. Nallur, S. Parimoo and S.M. Weissman. 1996. Genes in a 220-kb region spanning the TNF cluster in human MHC. *Genomics* 31:215-22 ; Neville, M.J. and R.D. Campbell. 1999. A new member of the Ig superfamily and a V-ATPase G subunit are among the predicted products of novel genes close to the TNF locus in the human MHC. *J. Immunol.* 162:4745-4754).

However, neither the function nor the cellular distribution of the putative product of IC7 gene was known and no indications existed on its role in natural cytotoxicity. In addition, the analysis of 1C7 transcript expression was limited to RT-PCR while no detection has been possible by Northern blot analysis. It should also be stressed that no correlation between transcript and surface expression could be established due to the lack of specific mAbs. In the present invention, we show that a precise correlation exists between the surface expression of NKp30, as determined by staining with three different mAbs, and mRNA expression, as assessed by Northern blot. On the contrary, the detection of 1C7 transcripts by RT-PCR does not allow predicting the surface expression of the 1C7/NKp30 molecule.

In conclusion, the NKp30 molecule represents a third member of an emerging family of receptors, termed Natural Cytotoxicity Receptors (NCR), that are involved in NK cell triggering upon recognition of non-HLA ligands. These receptors appear to complement each other in the induction of target cell lysis by NK cells. The relative contribution of each receptor is likely to reflect the expression/density of their specific ligands on target cells. Along this line, it

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on February 28, 2005.

Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 CFR §1.116
Examining Group 1644
Patent Application
Docket No. INN-103TD1
Serial No. 10/036,444

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Phuong N. Huynh
Art Unit	:	1644
Applicants	:	Alessandro Moretta, Cristina Bottino, Roberto Biassoni
Serial No.	:	10/036,444
Filed	:	January 7, 2002
Conf. No.	:	6065
For	:	Novel Triggering Receptor Involved in Natural Cytotoxicity Mediated by Human Natural Killer Cells and Antibodies That Identify the Same

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 C.F.R. §1.116

Sir:

In response to the final Office Action dated November 29, 2004, please amend the above-identified patent application as follows:

In the Claims

Claims 1-46 (Canceled).

Claim 47 (Previously Presented): A method for stimulation of cytotoxicity by NK cells, comprising:

contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, said antibody specifically binding to a polypeptide comprising the amino acid sequence of SEQ ID NO:2.

Claims 48-63 (Canceled).

Claim 64 (Previously Presented): The method according to claim 47, wherein said antibody is a polyclonal antibody.

Claim 65 (Previously Presented): The method according to claim 47, wherein said antibody is a monoclonal antibody.

Claim 66 (Previously Presented): The method according to claim 47, wherein said antibody is a humanized mouse monoclonal antibody.

Claim 67 (Previously Presented): The method according to claim 47, wherein said antibody is an antibody of human origin.

Claim 68 (Previously Presented): The method according to claim 65, wherein said monoclonal antibody is produced by the hybridoma having CNCM Registration Number I-2576.

Claims 69-73 (Canceled).

Claim 74 (Currently Amended): A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, said antibody being produced by the hybridoma having CNCM Registration Number I2576 and specifically binding to a polypeptide comprising the amino acid sequence of: SEQ ID NO: 4 or SEQ ID NO: 7.

Claims 75-79 (Canceled).

Claim 80 (Previously Presented): The method according to claim 74, wherein said antibody specifically binds to a polypeptide comprising SEQ ID NO: 4.

Claim 81 (Previously Presented): The method according to claim 74, wherein said antibody specifically binds to a polypeptide comprising SEQ ID NO: 7.

Claim 82 (Currently Amended): ~~The method according to claim 74~~ A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, wherein said antibody specifically binds to a polypeptide consisting of SEQ ID NO: 4.

Claim 83 (Currently Amended): ~~The method according to claim 74~~A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, wherein said antibody specifically binds to a polypeptide consisting of SEQ ID NO: 7.

Claim 84 (Previously Presented): A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody having the binding specificity of the antibody produced by the hybridoma having CNCM Registration Number I-2576, said antibody having the ability to stimulate the cytotoxicity of said NK cells.

Claim 85 (Currently Amended): A method of binding NK cells to antibody comprising contacting said NK cells with ~~an antibody~~ a monoclonal antibody produced by hybridoma CNCM Registration Number I-2576 or an immunoreactive fragment thereof, that specifically binds to the NKp30 polypeptide (SEQ ID NO: 2) or an immunogenic fragment thereof.

Claims 86-90 (Canceled).

Claim 91 (Previously Presented): The method according to claim 85, wherein said antibody or immunoreactive fragment thereof is coupled to a label.

Claim 92 (Previously Presented): The method according to claim 91, wherein said label is a fluorescent label.

Claim 93 (Previously Presented): The method according to claim 92, wherein said antibody or immunoreactive fragment thereof is attached to a solid support.

Claim 94 (Canceled).

Claim 95 (Currently Amended): The method according to claim 85, wherein said NK cells are contacted with immunoreactive fragment of ~~an antibody that specifically binds to the NKp30 polypeptide (SEQ ID NO: 2) or an immunoreactive fragment thereof~~ a monoclonal antibody produced by CNCM Registration Number I-2576.

Claim 96 (Canceled).

Remarks

Claims 47, 64-68 and 74, 80-85 and 91-93 and 95 are pending in the subject application. Claims 1-46, 48-63 and 69-73 are canceled. By this Amendment, Applicants have amended claims 74, 82-83, 85 and 95 and canceled claims 75-79, 86-90, 94 and 96. Support for the amended claims can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 47, 64-68, 74, 80-85, 91-93 and 95 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested. All reference to page and line numbers in this response relate to the substitute specification concurrently filed herewith.

As an initial matter, Applicants gratefully acknowledge the Examiner's indication that claims 47, 64-68 and 84 have been allowed. In addition, Applicants gratefully acknowledge the Examiner's indication that claims 79, 82, 83 and 90 would be allowable if re-written as independent claims. It is also respectfully submitted that claims 91-93 are also in condition for allowance as they depend from a claim that has been re-written in order to place it in allowable condition.

Claims 74-78, 80-81, 85-89 and 91-96 were rejected under 35 US §112, first paragraph. While Applicants respectfully disagree with this rejection, the cancellation of the claims has rendered the rejections moot. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 85-87, 91-92 and 95-96 are rejected under 35 U.S.C. §103(a) as being unpatentable over Biassoni *et al.* (Accession No. AJ223153 Sept. 1, 1999) in view of Harlow *et al.* (in Antibodies – A Laboratory Manual, 1988, Cold Spring Harbor Laboratory Publication, Cold Spring Harbor, NY, pages 92-94, 116-117 and 359-366), and Campbell *et al.* (in Monoclonal Antibody Technology, 1984, Elsevier Science Publisher, New York, NY, pages 1-32). Claims 88-89 are rejected under 35 U.S.C. §103(a) as being unpatentable over Biassoni *et al.* (Accession No. AJ223153, Sept. 1, 1999) in view of Harlow *et al.* (in Antibodies – A Laboratory Manual, 1988 Cold Spring Harbor Laboratory Publication, Cold Spring Harbor, NY, pages 92-94, 116-117 and 359-366), Campbell *et al.* (in Monoclonal Antibody Technology, 1984, Elsevier Science Publisher, New York, NY, pages 1-32), as applied to claims 85-87, 91-92 and 95-96 and further in view of U.S. Patent No. 5,530,101, filed

Dec. 1990). Claims 93-94 are rejected under 35 U.S.C. §103(a) as being unpatentable over Biassoni *et al.* (Accession No. AJ223153, Sept. 1, 1999) in view of Harlow *et al.* (in Antibodies – A Laboratory Manual, 1988 Cold Spring Harbor Laboratory Publication, Cold Spring Harbor, NY, pages 92-94, 116-117 and 359-366), Campbell *et al.* (in Monoclonal Antibody Technology, 1984, Elsevier Science Publisher, New York, NY, pages 1-32), as applied to claims 85-87, 91-92 and 95-96 and further in view of Ellison *et al.* (J. Immunological Methods 186:233-243; 1995). Applicants respectfully submit that the claims are not obvious over the cited combinations of references. However, in the interest of expediting prosecution in this matter Applicants have canceled these claims thereby rendering the rejections moot. Accordingly, reconsideration and withdrawal of the rejections is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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FCE/ssa

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on August 10, 2004.

Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 CFR §1.111
Examining Group 1644
Patent Application
Docket No. INN-103TD1
Serial No. 10/036,444

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Phuong N. Huynh
Art Unit	:	1644
Applicants	:	Alessandro Moretta, Cristina Bottino, Roberto Biassoni
Serial No.	:	10/036,444
Filed	:	January 7, 2002
Conf. No.	:	6065
For	:	Novel Triggering Receptor Involved in Natural Cytotoxicity Mediated by Human Natural Killer Cells and Antibodies That Identify the Same

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 CFR §1.111

Sir:

A Petition and Fee for a two-month Extension of Time through and including August 11, 2004, accompanies this Amendment.

In response to the Office Action dated March 11, 2004, please amend the above-identified patent application as follows:

APR 27 2006

In the Specification

Please replace the specification as-filed with the substitute specification enclosed with this Amendment.

In the Claims

Claims 1-46 (Canceled).

Claim 47 (Currently Amended): A method for stimulation of cytotoxicity by NK cells, comprising:

contacting said NK cells with an amount of antibody ~~or a binding fragment thereof~~ effective to stimulate the activity of said NK cells, said antibody ~~which~~ specifically ~~binds~~ binding to a polypeptide ~~having at least an~~comprising the amino acid sequence of SEQ ID NO:2~~, effective to stimulate their cytotoxicity~~.

Claims 48-63 (Canceled).

Claim 64 (Previously Presented): The method according to claim 47, wherein said antibody is a polyclonal antibody.

Claim 65 (Previously Presented): The method according to claim 47, wherein said antibody is a monoclonal antibody.

Claim 66 (Previously Presented): The method according to claim 47, wherein said antibody is a humanized mouse monoclonal antibody.

Claim 67 (Previously Presented): The method according to claim 47, wherein said antibody is an antibody of human origin.

Claim 68 (Currently Amended): The method according to claim 65, wherein said monoclonal antibody is produced by ~~hybridoma I-2576~~the hybridoma having CNCM Registration Number I-2576.

Claims 69-73 (Canceled)

Claim 74 (New): A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, said antibody specifically binding to a polypeptide comprising the amino acid sequence of: SEQ ID NO: 4 or SEQ ID NO: 7.

Claim 75 (New): The method according to claim 74, wherein said antibody is a polyclonal antibody.

Claim 76 (New): The method according to claim 74, wherein said antibody is a monoclonal antibody.

Claim 77 (New): The method according to claim 74, wherein said antibody is a humanized mouse monoclonal antibody.

Claim 78 (New): The method according to claim 74, wherein said antibody is an antibody of human origin.

Claim 79 (New): The method according to claim 76, wherein said monoclonal antibody is produced by the hybridoma having CNCM Registration Number I-2576.

Claim 80 (New): The method according to claim 74, wherein said antibody specifically binds to a polypeptide comprising SEQ ID NO: 4.

Claim 81 (New): The method according to claim 74, wherein said antibody specifically binds to a polypeptide comprising SEQ ID NO: 7.

Claim 82 (New): The method according to claim 74, wherein said antibody specifically binds to a polypeptide consisting of SEQ ID NO: 4.

Claim 83 (New): The method according to claim 74, wherein said antibody specifically binds to a polypeptide consisting of SEQ ID NO: 7.

Claim 84 (New): A method for stimulation of cytotoxicity by NK cells comprising contacting said NK cells with an amount of antibody having the binding specificity of the antibody produced by the hybridoma having CNCM Registration Number I-2576, said antibody having the ability to stimulate the cytotoxicity of said NK cells.

Claim 85 (New): A method of binding NK cells to antibody comprising contacting said NK cells with an antibody, or an immunoreactive fragment thereof, that specifically binds to the NKp30 polypeptide (SEQ ID NO: 2) or an immunogenic fragment thereof.

Claim 86 (New): The method according to claim 85, wherein said antibody is a polyclonal antibody.

Claim 87 (New): The method according to claim 85, wherein said antibody is a monoclonal antibody.

Claim 88 (New): The method according to claim 85, wherein said antibody is a humanized mouse monoclonal antibody.

Claim 89 (New): The method according to claim 85, wherein said antibody is an antibody of human origin.

Claim 90 (New): The method according to claim 85, wherein said monoclonal antibody is produced by hybridoma having CNCM Registration Number I-2576.

Claim 91 (New): The method according to claim 85, wherein said antibody or immunoreactive fragment thereof is coupled to a label.

Claim 92 (New): The method according to claim 91, wherein said label is a fluorescent label.

Claim 93 (New): The method according to claim 92, wherein said antibody or immunoreactive fragment thereof is attached to a solid support.

Claim 94 (New): The method according to claim 93, wherein said solid support is selected from the group consisting of paramagnetic microspheres, submicroscopic microbeads, semi-permeable substrate consisting of an array of hollow fibers, and dense particles.

Claim 95 (New): The method according to claim 85, wherein said NK cells are contacted with immunoreactive fragment of an antibody that specifically binds to the NKp30 polypeptide (SEQ ID NO: 2) or an immunogenic fragment thereof.

Claim 96 (New): The method according to claim 47, wherein said antibody cross-links NKp30 (SEQ ID NO: 2).

Remarks

Claims 47 and 60-73 are pending in the subject application. By this Amendment, Applicants have provided a substitute specification. Claims 1-46, 48-63 and 69-73 are canceled, claims 47 and 68 are amended, and claims 74-96 are newly added. Support for the new and amended claims can be found throughout the subject specification and in the claims as originally filed (see, for example, page 10, lines 17-26; page 13, lines 8-31; page 15, lines 15-30; and Example 1, pages 27-48). Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 47, 64-68 and 74-96 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested. All reference to page and line numbers in this response relate to the substitute specification concurrently filed herewith.

As an initial matter, Applicants gratefully acknowledge the Examiner's indication that Applicant should amend the first line of the specification to reflect the relationship between the instant application and application serial number 09/440,514 (filed November 15, 1999). Applicants note that this information was previously provided to the Examiner in a Preliminary Amendment dated May 19, 2003. For the convenience of the Examiner, a copy of this amendment is attached hereto and its entry is respectfully requested.

In addition, Applicants gratefully acknowledge the Examiner's indication that page 4, line 20, page 24, lines 12-27 and Figures 7B and 7C of the disclosure should be amended to correct typographical errors. By this Amendment, a substitute specification and new Figures 7B and 7C are being provided to correct these errors and a number of other typographical errors found throughout the specification. The undersigned avers that the substitute specification and replacement Figures contain no new matter. Entry of the new Figures and specification are respectfully requested. A red-lined copy of the specification indicating the changes made therein is also enclosed.

Claims 68 and 73 are rejected under 35 US §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully submit that antibodies having the binding specificity of AZ20, A76, and Z25 can be reproducibly obtained following the teachings of the specification. For example, the specification clearly teaches methods of making and screening both polyclonal and monoclonal antibodies (e.g.,

substitute specification at pages 26-32 and page 42). However, in the interest of advancing prosecution in this matter the undersigned has provided a declaration in this matter indicating that the AZ20 hybridoma has been deposited under the terms of the Budapest Treaty at the Collection Nationale de Cultures de Microorganisms, Institute Pasteur (CNCM), 25 rue de Docteur Roux, F-76724, Paris Cedex 15, France on November 8, 2000 and given CNCM Registration Number I-2576. This information has also been inserted into the specification (see substitute specification page 22, lines 24-28). Furthermore, all restrictions on this hybridoma will be irrevocably and without restriction or condition removed at the time a patent issues in this matter. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Claim 47 and 60-73 are rejected under 35 US §112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. Particularly, the Office Action argues that the specification is enabling only for a method of stimulating the cytotoxicity of NK cells with antibodies of human origin or a polyclonal, monoclonal, or humanized antibody that specifically binds to a polypeptide comprising SEQ ID NO: 2 or a peptide consisting of SEQ ID NOs: 4 or 7. The Office Action continues, arguing that the specification fails to enable: 1) methods of stimulating the cytotoxicity of NK cells with any antibody, or antibody binding fragment thereof, of human origin or a polyclonal, monoclonal, or humanized antibody that specifically binds to a polypeptide "having at least" an amino acid sequence of SEQ ID NOs: 2-7 wherein the antibody or fragment thereof is coupled to a label (e.g., a fluorescent label or a solid substrate such as a microspheres, hollow fibers, or dense particles). The Office Action then cites to *In re Wands* (858 F2d 731, 737; 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) and lists several factors that are indicated to be most relevant to this rejection. These factors include: 1) the scope of the claim; 2) the amount of guidance or direction provided; 3) the lack or sufficient working examples; 4) the unpredictability in the art; and the amount of experimentation required to enable one skilled in the art to practice the claimed invention. The Office Action further argues that claims 47 and 60-73 fail to comply with the written description requirement of 35 U.S.C. § 112, first paragraph on the grounds that the specification fails to provide adequate written description about the structure associated with the function of any polypeptide that contains additional undisclosed amino acids. Applicants traverse.

In view of the amendments made to the instant claims, it is respectfully submitted that many of the issues raised in the Office Action are now moot. However, Applicants traverse the rejection as applied to polypeptides comprising SEQ ID NO: 4 or 7 for methods of stimulating the cytotoxicity of NK cells.

As the Patent Office will note, the claims recite "A method for stimulation of cytotoxicity by NK cells, comprising: contacting said NK cells with an amount of antibody effective to stimulate the activity of said NK cells, said antibody specifically binding to a polypeptide comprising the amino acid sequence of: SEQ ID NO:2; SEQ ID NO: 4; or SEQ ID NO: 7." Thus, the scope of the claim indicates that the antibodies that are contacted with the NK cells specifically bind to SEQ ID NO: 2 or subsequences thereof (SEQ ID NOs: 4 or 7). The Patent Office further argues that the specification provides insufficient guidance with respect to the structure of any polypeptide to which an antibody binds and that given the indefinite number of undisclosed polypeptides, it is unpredictable which polypeptide is useful for making antibodies effective for the stimulation of NK activity. The Office Action goes on to cite a number of references in support of its assertion regarding the enablement of the subject application. The Office Action also argues that it is there is inadequate written description with respect to the structure of those polypeptides encompassed within the scope of the currently pending claims. It is respectfully submitted that the scope of such a claim is supported by the as-filed specification (*i.e.*, having adequate written description with respect to the claimed method and enabling the practice of the claimed invention) since it is taught that antibodies that specifically bind to SEQ ID NOs: 2 (the full length human NK p30 polypeptide), 4 (the extracellular domain of human NK p30), or 7 (an antigenic fragment of human NK p30). Indeed, polyclonal antibodies that were generated against the polypeptide of SEQ ID NO: 7 conjugated to KLH (an exemplary polypeptide comprising SEQ ID NO: 7 [see substitute specification page 33, lines 5-20]) are able to bind to and/or stimulate the cytotoxic activity of the NK cells (see Example 1). As the Patent Office will note, polyclonal antibodies were generated against SEQ ID NO: 7 which was conjugated to KLH specifically bound to NKp30 polypeptides. Furthermore, these polyclonal antibodies were able to immunoprecipitate polyclonal NK cell populations (see, for example, Figure 9 and pages 32-33 and 44-45 of the substitute specification). It is further submitted that adequate written description exists for polypeptides comprising SEQ ID

NO: 4 or 7 in that the "function" associated with these polypeptides is the binding of antibodies generated against these peptide fragments of SEQ ID NO: 2 or against the full-length sequence of SEQ ID NO: 2. Indeed, it is respectfully submitted that one skilled in the art would reasonably expect antibodies directed against SEQ ID NOs: 2, 4, or 7 to specifically bind to the NKp30 polypeptide and stimulate the cytotoxic activity of NK cells given the evidence provided in the as-filed application in Example 1 (namely that such antibodies immunoreact with the NKp30 polypeptide that is expressed on the surface of NK cells (see, for example Figures 3-7 and Figures 9A-B and the descriptions thereof at pages 22-27).

Applicants, further, respectfully submit that the specification, as filed, enables the breadth of the presently claimed invention. As the Patent Office is aware, the quantity of experimentation can be "considerable", "tedious", "laborious", and "time-consuming" as long as the experiments are merely "routine". See *Ex parte Jackson*, 217 U.S.P.Q. 804, 807 (B.P.A.I. 1982) ("[t]he test [of enablement] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine."); *Ex parte Erlich* 3 U.S.P.Q.2d 1011 (B.P.A.I. 1982) (observing that although a method might be "tedious and laborious," such experimentation is nevertheless "routine" defining "routine" experiments as those which use known methods in combination with the variables taught in the patent to achieve the expected, specific, patented result). In the case of the instant invention, Applicants submit that the specification provides explicit teachings regarding methods of making antibodies that stimulate the cytotoxicity of NK cells, methods of assaying NK cells to identify such antibodies, (substitute specification at pages 27-32) as well the criteria by which stimulatory antibodies according to the subject invention are identified (see substitute specification at pages 9-10, 27-32, Example 1, and Example 2 (pages 48-50)). Accordingly, reconsideration and withdrawal of the rejections is respectfully requested.

Previously presented claim 72 was rejected as constituting new matter in the previous Office Action. While this claim has been cancelled, additional new claims (see new claim 94) reciting this limitation have been presented in this response. Applicants submit that these claims are supported in the originally filed specification at, for example, page 10, about line 20 or pages 50-51 (Example 3) of the substitute specification. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 47, 60-63, 69-70, 72 and 73 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. It is respectfully submitted that these rejections are moot in view of the amendments made to the claims and withdrawal of the rejection is respectfully requested. Accordingly, reconsideration and withdrawal of the rejection under 35 US §112, second paragraph, is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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FCE/ssa

Enclosures: Substitute specification
Red-lined copy of specification
Replacement Figures 7B and 7C
Copy of Preliminary Amendment dated May 19, 2003
Declaration with attached copy of CNCM registration for deposit of hybridoma AZ20 under Budapest Treaty

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,979,546

APPLICATION NO.: 10/036,444

DATED : December 27, 2005

INVENTORS : Alessandro Moretta, Cristina Bottino, Roberto Biassoni

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 9,
Line 63, "anti-p44 antibodies" should read --anti-NKp44 antibodies--.

Column 13,
Line 25, "Fcy" should read --Fcγ--.

Column 18,
Line 60, "cuvefte" should read --cuvette--.

Column 22,
Line 35, "AZ**20** cell" should read --AZ20⁻ cell--.

Column 25,
Line 7, "EMBUGenBank" should read --EMBL/GenBank--.
Line 66, "NKp46 associated CD**34**ζ chain" should read
--NKp46-associated CD3ζ chain--.

Column 26,
Line 43, "(CD3ζFcεRIγ and" should read --(CD3ζ/FcεRIγ and--.
Line 61, "NKp46/NKp44 independent" should read --NKp46/NKp44-independent--.

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Line 21, "CD**34**ζ" should read --CD3ζ--.

Column 37,
Line 22, "NK cells wvith" should read --NK cells with--.
Line 23, "cffmdve" should read --effective--.
Line 25, "specificely" should read --specifically--.

Column 38,
Line 27, "Registrati" should read --Registration--.
Line 36, "fragnent" should read --fragment--.

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